BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Image Problem Mailbox.

The New England
Journal of Medicine

Volume 324 APRIL 4, 1991 Number 14

TRANSFORMING GROWTH FACTORS β1 AND α IN CHRONIC LIVER DISEASE

Effects of Interferon Alfa Therapy

ALBERTO CASTILLA, M.D., JESÚS PRIETO, M.D., AND NELSON FAUSTO, M.D.

Abstract *Background.* Cirrhosis is a diffuse process of hepatic fibrosis and regenerative nodule formation of unknown pathogenesis. Transforming growth factor (TGF) β1 induces the production of extracellular matrix proteins by liver cells and has been implicated in the pathogenesis of hepatic fibrosis in laboratory animals. TGFα is a hepatocyte mitogen that participates in liver regeneration.

Methods. Using Northern blot analysis, we studied the expression of TGFβ1 messenger RNA (mRNA) in liver specimens from 42 patients with chronic hepatitis and cirrhosis and 12 subjects with either normal or fatty livers. The results were correlated with measurements of procollagen Type I mRNA in liver tissue, procollagen Type III peptide in serum, and the degree of histologic injury. We also investigated whether TGFα mRNA would be detectable in biopsy specimens of livers with proliferative activity.

Results. TGFβ1 mRNA expression correlated closely with the expression of procollagen Type I mRNA (r = 0.94) and serum procollagen Type III peptide (r = 0.89) and with the histologic activity index (r = 0.73). All patients with increased fibrogenic activity (serum procollagen Type III peptide level, >11.9 μg per liter) had increased levels of TGFβ1 mRNA (2 to 14 times the levels in the control group or in patients with normal fibrogenic activity), and both TGFα and H3 histone (a marker of DNA synthesis) mRNAs were detectable in patients with regenerative nodules. Six of eight patients with hepatitis C treated with interferon alfa for one year had sustained clinical responses with normalization of serum procollagen Type III peptide and aminotransferase activity. All these patients had normal levels of TGFβ1 mRNA in liver specimens obtained at the end of the year.

Conclusions. TGFβ1 may have an important role in the pathogenesis of fibrosis in patients with chronic liver disease, and TGFα expression may be associated with liver regeneration in these patients. (N Engl J Med 1991; 324:933-40.)

FIBROSIS is an important component of advanced chronic inflammatory liver disease.[1-5] In cirrhosis, fibrosis is accompanied by cell necrosis and nodular regeneration.[2,3] Hepatic fibrosis is a complex process that involves changes in the amounts of extracellular matrix components, activation of cells capable of producing matrix materials, cytokine release, and tissue remodeling.[1,4-7]

In addition to being an inhibitor of DNA synthesis by hepatocytes,[8-12] transforming growth factor (TGF) β1 is associated with hepatic fibrosis.[13-15] TGFβ1 increases the production of extracellular matrix proteins and their receptors and inhibits the synthesis of matrix-degrading proteolytic enzymes.[16-20] In the liver, TGFβ1 induces collagen synthesis in lipocytes.[21-24] These cells, located in subendothelial spaces, are believed to become activated in hepatic fibrosis.[1,4,6,23-27] Using an experimental model of schistosomiasis in mice, Czaja et al.[13] found an increase in the content of TGFβ1 messenger RNA (mRNA) in the liver of infected animals, in parallel with an increase in Type I procollagen mRNA. Nakatsukasa et al.[24] also found a positive correlation between TGFβ1 mRNA and procollagen I, III, and IV mRNAs in the livers of rats with carbon tetrachloride–induced hepatic fibrosis. In view of these observations, we wondered whether the expression of TGFβ1 mRNA would correlate with various indicators of liver fibrosis and injury in humans. We therefore measured the steady-state levels of TGFβ1 mRNA in liver biopsy specimens from patients with chronic hepatitis and cirrhosis and correlated its expression with that of procollagen Type I mRNA in the same biopsy specimen and with serum levels of procollagen Type III peptide. To determine the extent of proliferative activity in the livers of patients with chronic hepatic disease, we measured the expression of H3 histone mRNA, generally considered a good marker of DNA synthesis.[28-30] Since TGFα is a potent hepatocyte mitogen produced in vivo and in vitro by hepatocytes stimulated to proliferate,[9,31]

From the Center for Biomedical Research, Department of Internal Medicine, Clínica Universitaria, University of Navarra, Pamplona, Spain (A.C., J.P.), and the Department of Pathology and Laboratory Medicine, Division of Biology and Medicine, Brown University, Providence, R.I. (A.C., N.F.). Address reprint requests to Dr. Fausto at the Department of Pathology, Box G-B518, Brown University, Providence, RI 02912.

Supported by Beca Sandoz 1988 from the Spanish Society of Internal Medicine and Beca PF90 16247035 from the Ministerio de Educación y Ciencia of Spain (to A. Castilla), a grant from the Fundación Ramon Areces (to Dr. Prieto), and grants (CA-23226 and CA-35249) from the National Cancer Institute (to Dr. Fausto).

we analyzed the correlation between the expression of H3 histone mRNA and TGFα mRNA in chronic liver disease. Finally, in a small group of patients with chronic hepatitis C, we determined whether treatment with interferon alfa reduced the fibrogenic and proliferative activity of the disease and the expression of TGFβ1 mRNA in the liver.

METHODS

Study Groups

Liver biopsy specimens were obtained from 46 patients with elevated serum alanine aminotransferase (ALT) activity who had either clinical features suggesting chronic liver disease or a previous diagnosis of such disease. Specimens were also taken from eight patients with normal results of liver-function tests who had undergone cholecystectomy for cholelithiasis, with no clinical complications. The protocol for this study was approved by the ethics committee of the University of Navarra (Pamplona, Spain), and informed consent was given by all patients. The specimens (minimal size, 16 mm long) were obtained with a TruCut biopsy needle. Approximately half of each specimen was immediately frozen in liquid nitrogen and stored at −80°C until it was used for RNA extraction; the rest of the specimen was processed for histologic study according to standard procedures.

The specimens of 4 of the 46 patients showed fatty liver but no fibrosis or necrosis. These 4 patients together with the 8 with normal liver function constituted the control group (7 women and 5 men, ranging in age from 29 to 51 years; mean age, 46). The 42 patients with hepatic fibrosis or necrosis (20 with chronic hepatitis and 22 with cirrhosis) included 16 women and 26 men, ranging in age from 21 to 62 years (mean, 42). This group of 42 patients was divided into three subgroups as follows.

Chronic Hepatitis B Infection

Eleven patients (three women and eight men, ranging in age from 21 to 61 years; mean age, 34) had positive tests for at least six months for serum hepatitis B surface antigen and anti–core antibody and negative tests for antibody against hepatitis B surface antigen. In three of these patients, markers for viral replication (hepatitis B virus e antigen, viral DNA, and viral DNA-polymerase activity) were present in serum; in the other eight, these markers were absent and anti-e antibody was present. All patients were negative for anti-delta antibody.

Chronic Hepatitis C Infection

Twenty-nine patients (12 women and 17 men, ranging in age from 21 to 62 years; mean age, 45) had elevated serum levels of ALT activity for at least six months and a diagnosis of chronic hepatitis (14 patients) or cirrhosis (15 patients) based on liver biopsy. All had a history of parenteral exposure to hepatitis C virus and a positive serum test for anti–hepatitis C antibodies. None of the patients had detectable serum hepatitis B surface antigen or antinuclear, anti–smooth muscle, or mitochondrial antibodies, and all had normal serum levels of copper, iron, transferrin, ceruloplasmin, and α_1-antitrypsin. Likewise, none had drug- or alcohol-induced liver disease.

Nonviral Chronic Liver Disease

Two patients had nonviral chronic liver disease. They were a 50-year-old woman with primary biliary cirrhosis (antimitochondrial antibody titer, 1/320; histologic stage IV) and a 21-year-old man with active cirrhosis associated with ulcerative colitis (viral markers and autoantibodies were absent).

None of the patients in the three groups had serum antibodies to human immunodeficiency virus Type 1 (HIV-1).

Interferon Treatment

Eight patients (four with chronic hepatitis and four with cirrhosis; two women and six men, ranging in age from 21 to 59 years; mean age, 37) from the subgroup with hepatitis C were studied after one year of treatment with interferon alfa-n1 (lymphoblastoid interferon; Wellferon, Galloso–Wellcome, Madrid). These patients were treated in a randomized trial that included a total of 36 patients. Although liver specimens were obtained from the eight patients before and after treatment, RNA could be measured only in the final biopsy specimen since no samples had been obtained for RNA extraction at the time of the original biopsy. Interferon was given according to the following schedule.

Induction Phase

Daily doses of 3 million units were given until serum ALT levels decreased to less than 50 U per liter (upper limit of normal range, 40) and remained stable for two weeks. If this failed to occur within two months after the start of treatment, the daily dose was increased to 4.5 million units. This dose was given until the serum ALT levels decreased to less than 50 U per liter and remained at this concentration for a two-week period, or for two months if the serum ALT level did not fall to less than 50 U per liter. At the end of the induction phase, seven of the eight patients had serum ALT levels in the normal range.

Maintenance Phase

All eight patients (including the patient who had not responded to treatment during the induction phase) were treated with 3 million units of interferon three times a week for 3 months, followed by doses of 1.5 million units three times a week, for a total of 12 months of treatment (induction and maintenance phases).

During the one-year treatment period, six of the eight patients had normal serum ALT levels. One patient had a transient response (normal serum ALT levels for several months, followed by reactivation of liver disease and abnormal ALT levels). The patient who did not have a response to interferon during the induction phase also had no response (no change in serum ALT level) during the rest of the treatment period.

RNA Extraction and Northern Blot Analysis

RNA was isolated from frozen liver tissue according to a modification of the Chirgwin procedure.[8,31,33] The liver specimens were homogenized in 4 M guanidinium thiocyanate solution. After low-speed centrifugation to remove cellular debris, the samples were layered on a 3.5-ml cushion of 5.7 M cesium chloride in 25 mM sodium acetate (pH 5). They were then centrifuged for 20 hours at 20,000 rpm in a Beckman SW40 Ti rotor. The RNA was dissolved in water and recovered by ethanol precipitation. RNA content was measured by spectrophotometry (absorbance at 260 nm).

RNA samples (10 μg per lane) were fractioned by electrophoresis in 6.2 percent formaldehyde–1.1 percent agarose gels and transferred to nitrocellulose filters in 20× SSC (1× SSC is 0.15 M sodium chloride–0.015 M trisodium citrate). To ensure that similar amounts of RNA were loaded into the various gel lanes, the quantity of 18S and 28S RNA was determined by ethidium bromide fluorescence staining before RNA transfer. The blots were then hybridized at 42°C for 72 hours with ^{32}P-labeled complementary DNA probes for human TGFβ1,[8] human TGFα (925-bp *Eco*RI fragment of human prepro-TGFα, provided by Dr. G.I. Bell), human α_1(I) procollagen[34] (clone Hf677, provided by Dr. K. Zaret), murine H3 histone gene (*Sal*I–*Bam*HI 204-bp fragment from histone 3.2, provided by Dr. L. Biempica), and rat glyceraldehyde-3-phosphate dehydrogenase[35,36] (provided by Dr. R. Wu). All probes were labeled by nick translation.[8] After washing at 50°C, the filters were exposed to Kodak XAR-2 film at −70°C with intensifying screens. The autoradiographs were quantitated by scanning densitometry with a Gilford spectrophotometer. The expression of α_1(I) procollagen mRNA was determined in only 34 patients because of technical problems with the hybridization procedure in one of the filters.

The expression of glyceraldehyde-3-phosphate dehydrogenase mRNA was used as the internal control for each specimen. The content of TGFβ1 mRNA (densitometry absorbance after 96 hours of autoradiographic exposure), TGFα mRNA (192 hours of exposure), procollagen I mRNA (24 hours of exposure), and H3 histone mRNA (192 hours of exposure) was normalized to that of glyceral-

dehyde-3-phosphate dehydrogenase mRNA (2 hours of exposure) in the same biopsy sample.

Amino-Terminal Peptide of Procollagen Type III

Serum procollagen III amino-terminal peptide was measured with a commercial radioimmunoassay kit (RIA-gnost P-III-P; Behringwerke AG, Marburg, Germany). Normal values (mean ±SD, 9.9±1.0 μg per liter) were established from serum measurements in 12 normal subjects (4 women and 8 men, ranging in age from 29 to 49 years; mean age, 39).

Viral Markers

Commercially available radioimmunoassay kits were used to detect hepatitis B surface and e antigens; antibodies to the surface, e, and core antigens; and anti–hepatitis delta antibodies (AUSRIA II, AUSAB, CORAB, HBe kit, and anti-Delta kit; Abbott Laboratories, North Chicago). Serum levels of hepatitis B virus DNA and DNA-polymerase activity were measured by dot hybridization and enzymatic reaction as previously reported.[37,38] Antibodies against hepatitis C and HIV-1 were determined by enzyme-linked immunosorbent assay (Ortho, Raritan, N.J., and Abbott Laboratories, respectively).

Liver Biopsies

Biopsy specimens were graded for their degree of periportal, portal, and lobular inflammation and fibrosis according to the scoring system of Knodell et al.[7] Coded specimens were examined by a pathologist who had no knowledge of their source.

Statistical Analysis

The results are presented as means ±SD. The results were evaluated by analysis of variance, polynomial regression, the Mann–Whitney test, the Wilcoxon signed-rank test, and Fisher's exact test.

Results

TGFβ1 mRNA Expression and Fibrogenesis

We first determined whether the various indicators of fibrogenesis correlated with each other in the 42 patients with chronic hepatitis or cirrhosis. The expression of procollagen Type I mRNA in liver specimens on Northern blot analysis correlated directly with the serum levels of procollagen Type III amino-terminal peptide ($r = 0.91$, $P<0.001$) (Fig. 1A). Furthermore, the serum levels of procollagen Type III peptide correlated significantly with the serum levels of ALT ($r = 0.84$, $P<0.001$) (Fig. 1B). We then measured the expression of TGFβ1 mRNA and procollagen Type I mRNA in each specimen from the patients with chronic hepatic disease and the control group (Fig. 2). To eliminate variations that might be caused by technical procedures, the levels of TGFβ1 mRNA and procollagen Type I mRNA in each patient were calculated in relation to the level of glyceraldehyde-3-phosphate dehydrogenase in the same sample (Fig. 2). An analysis of the results in all patients revealed that the expression of TGFβ1 in the biopsy specimens correlated strongly with that of liver procollagen Type I mRNA ($r = 0.94$, $P<0.001$; $n = 32$) and with the serum levels of procollagen Type III peptide ($r = 0.89$, $P<0.001$) (Fig. 3). Liver concentrations of TGFβ1 mRNA and serum concentrations of procollagen Type III peptide were low in the control patients (mean procollagen Type III peptide concentration, 10.0±0.3 μg per liter) but varied widely in the patients with chronic liver disease (Fig. 3).

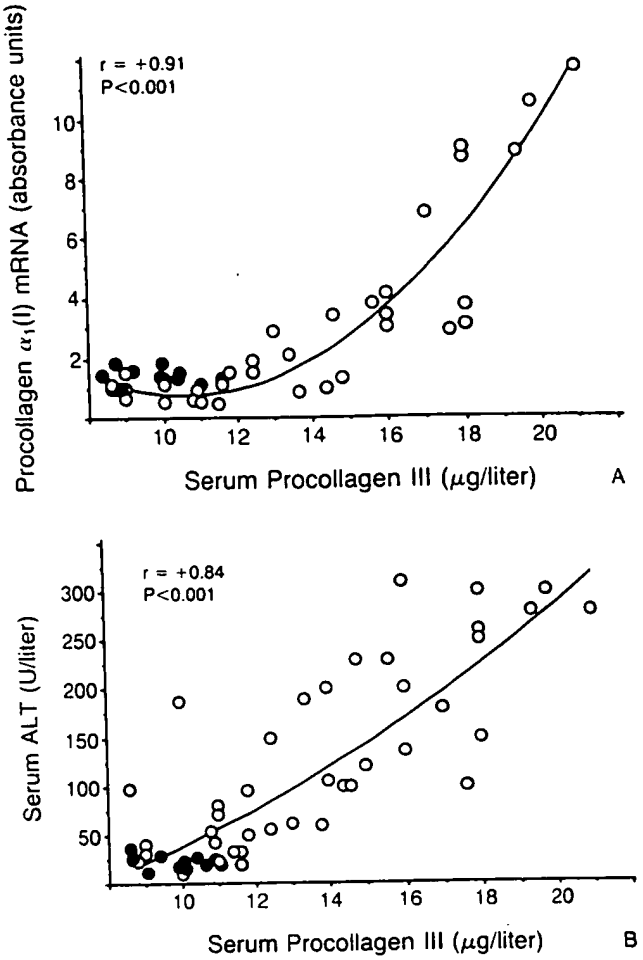

Figure 1. Serum Concentrations of Procollagen Type III Peptide in Relation to Hepatic Procollagen α_1(I) mRNA Expression (A) and Serum ALT Activity (B) in Patients with Chronic Liver Disease (○) and Control Patients (●).

In Panel A, the procollagen α_1(I) mRNA content in 34 patients and 12 control patients has been normalized to that of glyceraldehyde-3-phosphate dehydrogenase mRNA as described in Methods, and expressed in densitometric absorbance units. The mean (±SD) serum procollagen III values in the control group averaged 10.0±0.3 μg per liter (range, 8.6 to 11.6).

Panel B shows serum ALT activity in 40 patients with chronic liver disease and the 12 control patients. The mean (±SD) value in the control group was 25±4 U per liter (range, 18 to 32).

To analyze in more detail the relation between TGFβ1 mRNA expression and fibrogenesis, the patients were divided into two groups according to the serum level of procollagen Type III peptide: those with high fibrogenic activity (serum procollagen Type III peptide >11.9 μg per liter, corresponding to 2 SD above the mean in the normal subjects) and those with normal fibrogenic activity. The patients with high fibrogenic activity had increased levels of TGFβ1 mRNA, ranging from 2 to 14 times above the amounts found in the control group, whereas the patients with normal fibrogenic activity had TGFβ1 mRNA levels

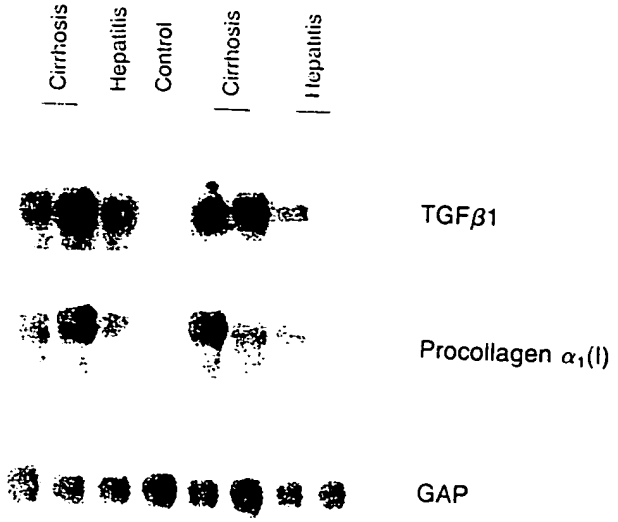

Figure 2. Representative Northern Blot Analysis of TGFβ1, Procollagen α_1(I), and Glyceraldehyde-3-Phosphate Dehydrogenase (GAP) mRNAs in Liver Specimens from Control Patients, Patients with Chronic Hepatitis, and Patients with Cirrhosis.

that did not differ from the level in the control group (Fig. 4). Although there was considerable variation, the highest levels of hepatic TGFβ1 mRNA were detected in patients with cirrhosis who had high fibrogenic activity.

TGFβ1 mRNA Expression and Tissue Injury

The histologic activity index described by Knodell et al.[39] can be used as a semiquantitative method to assess the degree of histologic injury in chronic liver disease. For each patient, the scores for the total index and some subindexes were compared with the levels of TGFβ1 mRNA in the same biopsy sample. TGFβ1 mRNA expression correlated directly with the total index ($r = 0.73$, $P<0.001$) and some subindexes (piecemeal necrosis: $r = 0.67$, $P<0.001$; fibrosis: $r = 0.69$, $P<0.001$; lobular injury $r = 0.54$, $P<0.005$; and portal inflammation: $r = 0.53$, $P<0.01$).

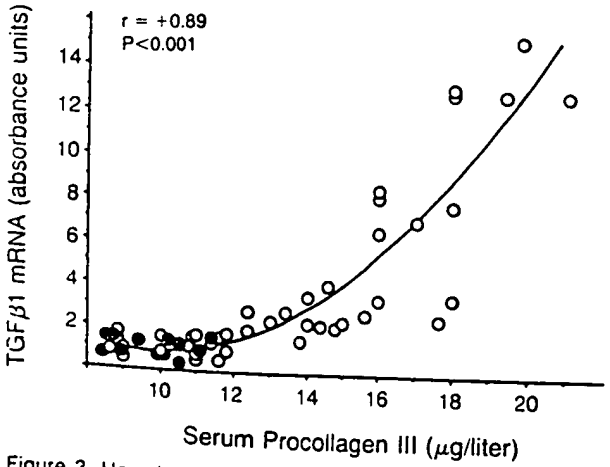

Figure 3. Hepatic TGFβ1 mRNA Expression in Relation to the Serum Concentration of Procollagen Type III Peptide in 42 Patients with Chronic Liver Disease (C) and 12 Control Patients (●). TGFβ1 mRNA content was normalized to that of glyceraldehyde-3-phosphate dehydrogenase mRNA and expressed in densitometric absorbance units.

Expression of H3 Histone and TGFα mRNAs in Chronic Liver Disease

To assess the level of proliferative activity in the livers of the 42 patients and the control group, we examined the expression of H3 histone gene mRNA in liver specimens. H3 mRNA was detectable by Northern blot analysis of specimens (Fig. 5) in all 22 patients with cirrhosis, but in only 11 of 20 patients (55 percent) with chronic hepatitis ($P<0.01$ by Fisher's exact test). H3 mRNA was also detected in 8 of the 12 control patients, although in very low amounts (Fig. 5).

The pattern of expression of TGFα mRNA was similar to that of H3 histone (Fig. 5). TGFα mRNA was detectable in all patients with cirrhosis and in 60 percent of those with chronic hepatitis ($P<0.01$ by Fisher's exact test) and was present in very low

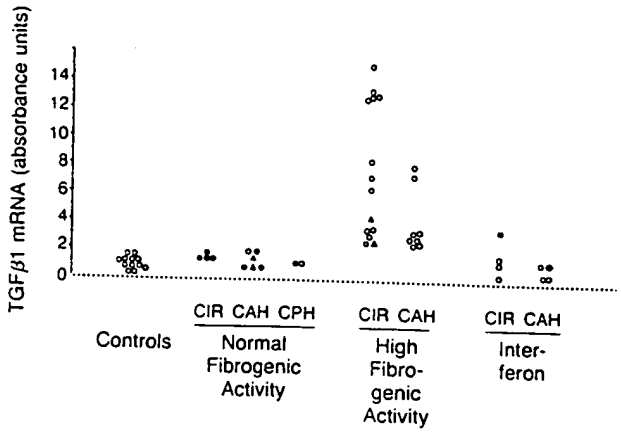

Figure 4. Hepatic TGFβ1 mRNA Expression in Control Patients and Patients with Chronic Liver Disease Untreated or Treated with Interferon.

The patients with chronic liver disease were divided into three groups: those with high fibrogenic activity (a serum procollagen Type III peptide concentration higher than 11.9 μg per liter, corresponding to the mean +2 SD in normal subjects), those with normal fibrogenic activity, and those with chronic hepatitis C virus infection and high fibrogenic activity who were treated with interferon for one year. For each patient, TGFβ1 mRNA content was normalized to that of glyceraldehyde-3-phosphate dehydrogenase mRNA, as described in Methods. CIR denotes cirrhosis, CAH chronic active hepatitis, and CPH chronic persistent hepatitis. The interferon-treated group included six patients who responded to treatment (○), one patient who had a transient response (*), and one patient who had no response (■). The other two groups included patients with infection with hepatitis C virus (○), infection with hepatitis B virus and positive (▲) or negative (●) status for viral DNA in serum, primary biliary cirrhosis (□), and cirrhosis associated with ulcerative colitis (△).

The mean value for TGFβ1 expression in the group of patients with high fibrogenic activity who were not treated with interferon was significantly higher than the mean in the other three groups shown ($P<0.001$).

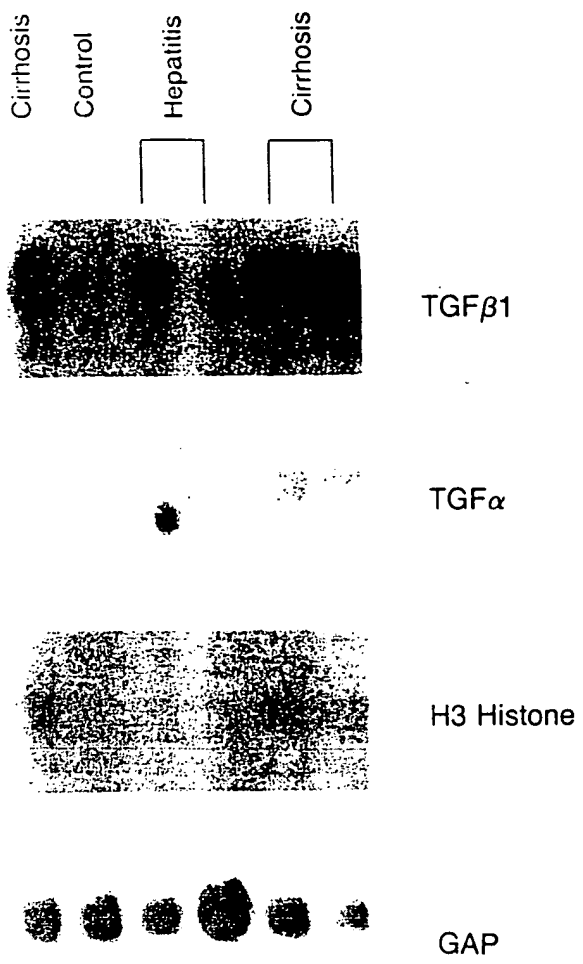

Figure 5. Representative Northern Blot Analysis of TGFβ1, TGFα, and H3 Histone mRNAs in Liver Specimens.

TGFα and H3 mRNAs were detected in patients with cirrhosis but were barely detectable in patients with chronic hepatitis or control patients. The expression of glyceraldehyde-3-phosphate dehydrogenase (GAP) mRNA is shown for comparison.

amounts in 4 of the 12 control patients. However, the intensity of the TGFα bands in the Northern blots was lower than that of the H3 histone mRNA (Fig. 5) and close to the limit of sensitivity of the method. Thus, although the determinations of H3 histone mRNA in all patients could be used for quantitative analysis, quantitation of TGFα mRNA was possible in only 16 patients. In these samples the expression of H3 histone mRNA correlated with that of TGFα mRNA ($r = 0.70$, $P<0.01$; data not shown).

Effect of Interferon Treatment

TGFβ1 mRNA Expression

Eight patients with chronic hepatitis C virus infection (cirrhosis or chronic hepatitis) and high fibrogenic activity were treated for one year with interferon as described in Methods. Six of these patients (Fig. 4) had a sustained clinical response to treatment, with normalization of serum procollagen Type III peptide levels (concentration before vs. after therapy, 16.4±4.0 vs. 9.3±1.3 μg per liter; $P<0.05$ by Wilcoxon's signed-rank test) and serum ALT activity. In all six of these patients, hepatic TGFβ1 mRNA expression was in the normal range at the end of the treatment period and did not differ from expression in untreated patients with normal fibrogenic activity or the control patients. TGFβ1 mRNA expression was normal in one patient who had only a transient response to interferon (indicated by the asterisk in Fig. 4), but it remained high in the patient who did not respond to the treatment (indicated by the square in Fig. 4; the serum level of Type III peptide in this patient was 16.0 μg per liter after therapy). Representative Northern blot analyses of TGFβ1 mRNA in liver specimens of interferon-treated and untreated patients are shown in Figure 6. The expression of the mRNA was much lower in the treated and the control patients than in the untreated patients, with the exception of one patient with chronic hepatitis and normal fibrogenic activity, who had low levels of TGFβ1 mRNA (third lane from left, Fig. 6).

H3 Histone mRNA Expression and Tissue Injury

The expression of H3 histone mRNA in the patients treated with interferon was similar to that in the control patients (Fig. 7). This was true not only of the six patients who responded to interferon but also of the two who had a transient response or no response to treatment (indicated by the asterisk and square in Fig.

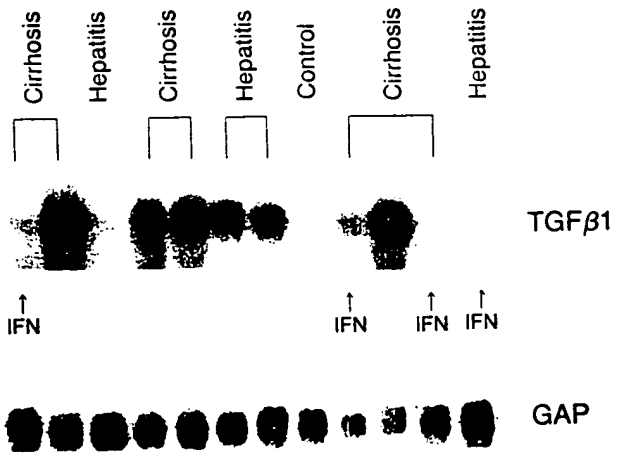

Figure 6. Representative Northern Blot Analysis of TGFβ1 mRNA in Liver Specimens from Patients with Chronic Liver Disease Untreated or Treated with Interferon.

Blots are shown for a control patient, patients with cirrhosis, patients with chronic hepatitis, and patients infected with hepatitis C virus treated with interferon (IFN) for one year. Note that in the treated patients, TGFβ1 mRNA expression was much lower than in untreated patients, with the exception of one untreated patient with chronic hepatitis and normal fibrogenic activity (third lane from left; a member of the group with normal fibrogenic activity shown in Fig. 4). The expression of glyceraldehyde-3-phosphate dehydrogenase (GAP) mRNA is shown for comparison.

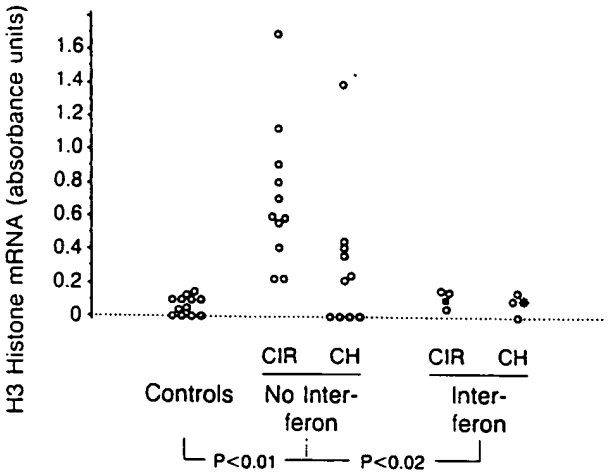

Figure 7. Effect of Interferon Therapy on Hepatic H3 Histone mRNA Expression in Patients with Chronic Hepatitis C Virus Infection.

The H3 mRNA content of each specimen was normalized to that of glyceraldehyde-3-phosphate dehydrogenase mRNA, as described in Methods.

The symbols represent the same patients shown in Figure 4. CIR denotes cirrhosis, and CH chronic hepatitis.

7). Portal and lobular inflammation and the degree of histologic damage decreased (Table 1) in the seven patients who had some response to treatment (six patients with a sustained response and one with a transient response).

Discussion

Although the changes in liver architecture that occur in cirrhosis have been described in detail, the pathogenesis of fibrosis in this process is largely unknown. Collagens Type I and III, present in approximately equal amounts, constitute about 80 percent of the total collagen content of the normal human liver.[1,4-7] In fibrosis, the amounts of collagen increase, but the increases in Type I collagen are generally greater than those in Type III, increasing the ratio of Type I to Type III twofold to fourfold.[1,4,5] It is not known whether these changes are due to increased collagen synthesis, decreased collagen degradation, or both.

Table 1. Changes in the Histologic Activity Index and Subindexes in Patients with Chronic Hepatitis C Virus Infection Who Responded to Therapy with Interferon.*

Index	Before Therapy	After Therapy
Histologic activity index	12.4±3.2	5.7±2.0†
Subindexes		
Portal inflammation	3.7±0.5	2.6±0.8†
Piecemeal necrosis	4.1±1.6	0.85±1.2†
Lobular injury	2.6±1.1	0.71±0.5†
Fibrosis	2.3±0.9	1.6±1.1

*Values are means ±SD in seven of eight patients with chronic hepatitis C virus infection; the scores did not change in the patient who did not respond to treatment. Indexes were calculated according to the method of Knodell et al.[39]

†P<0.05 by Wilcoxon's signed-rank test.

The expression of procollagen Type I mRNA in the liver is a good indicator of fibrogenic activity.[7,10] The serum level of procollagen Type III peptide also serves as a marker for liver fibrogenesis, although some peptide may be derived from collagen degradation.[1,4,7,41] We found that the expression of TGFβ1 mRNA in the liver correlated directly with both the hepatic level of procollagen Type I mRNA and the serum level of collagen Type III peptide. In patients with increased fibrogenic activity (indicated by high serum levels of procollagen Type III peptide), the expression of hepatic TGFβ1 mRNA was 2 to 14 times higher than that in the control patients. These results indicate that collagen synthesis is an important component of fibrogenesis in chronic liver disease, and are in agreement with the work of Annoni et al.[42] Furthermore, the results suggest that TGFβ1 may play a part in the pathogenesis of fibrosis in chronic hepatitis and cirrhosis.

In cultured cells, TGFβ1 stimulates the synthesis of collagens and other extracellular matrix components and retards their degradation.[16-20] In addition, TGFβ1 is capable of activating lipocytes, cells that are probably a major site of synthesis of matrix proteins in chronic liver disease.[1,4,6,7,21-24,43-49] A parallel increase in hepatic TGFβ1 and procollagen mRNAs has been found in experimental models of liver fibrosis.[21,24] In particular, Nakatsukasa et al.[24] found increased levels of TGFβ1 mRNA in lipocytes, myofibroblasts, and fibroblasts in rats with carbon tetrachloride–induced liver fibrosis. In the early stages of fibrosis, TGFβ1 mRNA was also detected in inflammatory cells infiltrating the liver. Similar studies are necessary to determine which cells may be the sources of TGFβ1 mRNA in the livers of patients with chronic hepatitis or cirrhosis. Nevertheless, it is reasonable to speculate that in the development of cirrhosis, TGFβ1 produced by lipocytes and perhaps other nonparenchymal cells induces collagen synthesis in these same cells.

A substantial proportion of patients with chronic hepatitis C have improved during treatment with interferon alfa.[50-53] Although the response to treatment was sometimes transient, the lobular and periportal inflammation of the liver regressed in many patients. As part of a larger study of the effects of this form of interferon in patients with chronic hepatitis C,[32] we determined the amount of TGFβ1 mRNA and procollagen Type I mRNA in liver specimens from patients treated with interferon for one year. Patients who responded to treatment had normalization of the biochemical indicators of fibrogenesis (serum level of procollagen Type III peptide and expression of Type I procollagen mRNA in the liver) and normal amounts of liver TGFβ1 mRNA at the end of treatment. Although the number of patients studied was small, the results suggest that treatment with inter-

feron decreases fibrogenesis. In this and other studies,[30,32,33] the histopathological score for liver fibrosis changed little during treatment although the scores for portal inflammation, piecemeal necrosis, and lobular injury decreased. Perhaps the degradation and resorption of fibrous tissue requires more than 12 months, as it does in patients with cirrhosis treated with colchicine.[34] We do not know whether the effect of interferon on Type I procollagen mRNA is a consequence of its action on TGFβ1 expression or whether the effect depends on the inhibition of viral replication. In experimental models of liver fibrosis that did not involve viral infection, interferon gamma decreased the expression of procollagen I and III mRNAs in liver cells in a dose-dependent manner, both in vivo and in vitro.[35]

TGFα mRNA was detected in the livers of all patients with regenerative nodules, but only in some patients with chronic hepatitis but no cirrhosis. In addition, the expression of TGFα mRNA closely correlated with that of H3 histone mRNA, a good indicator of cell proliferation.[28-30] These findings suggest that in patients with cirrhosis, TGFα is associated with hepatic-cell proliferation as in liver regeneration in rats after partial hepatectomy.[31] Hepatocellular necrosis is an important initiating event in both extracellular matrix deposition and cell proliferation (regeneration) in cirrhosis.[2,4,9,27] In patients with chronic hepatitis C who had a sustained response to interferon treatment, serum ALT activity and H3 histone mRNA expression were normal.

In summary, TGFβ1 may have an important role in fibrogenesis in chronic hepatitis and cirrhosis, and interferon therapy in patients with chronic hepatitis C can decrease the expression of TGFβ1 mRNA and procollagen Type I mRNA in the liver. Furthermore, we suggest that TGFα may be associated with cell proliferation in patients with chronic liver disease.

We are indebted to Ms. Deborah A. Martin, Ms. Elizabeth Ko, and Ms. Carol White (Brown University, Providence, R.I.) for technical support; to Dr. Miguel A. Muñoz and Dr. Joan Camps (Clínica Universitaria, Pamplona, Spain) and Dr. Miguel A. Simon (Social Security Hospital, Logroño, Spain) for collaboration in clinical work; and to Drs. G.I. Bell, K. Zaret, L. Biempica, J. Tso, X.-H. Sun, and R. Wu for the gift of various probes.

References

1. Bissell DM, Roll J. Connective tissue metabolism and hepatic fibrosis. In: Zakim D, Boyer TD, eds. Hepatology: a textbook of liver disease. 2nd ed. Philadelphia: W.B. Saunders, 1990:424-44.
2. Sherlock S. Hepatic cirrhosis. In: Sherlock S, ed. Diseases of the liver and biliary system. 8th ed. Oxford, England: Blackwell Scientific, 1989:410-24.
3. Conn HO, Atterbury CE. Cirrhosis. In: Schiff L, Schiff ER, eds. Diseases of the liver. Philadelphia: J.B. Lippincott, 1987:725-864.
4. Hahn EG, Schuppan D. Pathogenic mechanisms: fibrosis, fibrogenesis and fibrolysis. In: Tygstrup N, Orlandi F, eds. Cirrhosis of the liver: methods and fields of research. Amsterdam: Elsevier, 1987:63-81.
5. Rojkind M, Greenwel P. The liver as a bioecological system. In: Arias IM, Jakoby WB, Popper H, Schachter D, Shafritz DA, eds. The liver: biology and pathobiology. 2nd ed. New York: Raven Press, 1988:1269-85.
6. Bissell M. Cell-matrix interactions and hepatic fibrosis. In: Popper H, Schaffner F, eds. Progress in liver diseases. Vol. 9. Philadelphia: W.B. Saunders, 1990:143-55.
7. Biagini G, Ballardini G. Liver fibrosis and extracellular matrix. J Hepatol 1989; 8:115-24.
8. Braun L, Mead JE, Panzica M, Mikumo R, Bell GI, Fausto N. Transforming growth factor β mRNA increases during liver regeneration: a possible paracrine mechanism of growth regulation. Proc Natl Acad Sci U S A 1988; 85:1539-43.
9. Fausto N, Mead JE. Regulation of liver growth: protooncogenes and transforming growth factors. Lab Invest 1989; 60:4-13.
10. Nakamura T, Tomita Y, Hirai R, Yamaoka K, Kaji K, Ichihara A. Inhibitory effect of transforming growth factor-β on DNA synthesis of adult rat hepatocytes in primary culture. Biochem Biophys Res Commun 1985; 133:1042-50.
11. Carr BI, Hayashi I, Branum EL, Moses HL. Inhibition of DNA synthesis in rat hepatocytes by platelet-derived type β transforming growth factor. Cancer Res 1986; 46:2330-4.
12. McMahon JB, Richards WL, del Campo AA, Song MK, Thorgeirsson SS. Differential effects of transforming growth factor-β on proliferation of normal and malignant rat liver epithelial cells in culture. Cancer Res 1986; 46:4665-71.
13. Czaja MJ, Weiner FR, Flanders KC, et al. In vitro and in vivo association of transforming growth factor-β1 with hepatic fibrosis. J Cell Biol 1989; 108:2477-82.
14. Armendariz-Borunda J, Seyer JM, Kang AH, Raghow R. Regulation of TGFβ gene expression in rat liver intoxicated with carbon tetrachloride. FASEB J 1990; 4:215-21.
15. Nakatsukasa H, Evarts RP, Hsia C-C, Thorgeirsson SS. Transforming growth factor β1 and Type I procollagen transcripts during regeneration and early fibrosis of rat liver. Lab Invest 1990; 63:171-80.
16. Ignotz RA, Massagué J. Transforming growth factor-β stimulates the expression of fibronectin and collagen and their incorporation into the extracellular matrix. J Biol Chem 1986; 261:4337-45.
17. Roberts AB, Sporn MB, Assoian RK, et al. Transforming growth factor type β: rapid induction of fibrosis and angiogenesis in vivo and stimulation of collagen formation in vitro. Proc Natl Acad Sci U S A 1986; 83:4167-71.
18. Raghow R, Postlethwaite AE, Keski-Oja J, Moses HL, Kang AH. Transforming growth factor-β increases steady state levels of type I procollagen and fibronectin messenger RNAs posttranscriptionally in cultured human dermal fibroblasts. J Clin Invest 1987; 79:1285-8.
19. Edwards DR, Murphy G, Reynolds JJ, et al. Transforming growth factor β modulates the expression of collagenase and metalloproteinase inhibitor. EMBO J 1987; 6:1899-904.
20. Roberts AB, Sporn MB. The transforming growth factor-βs. In: Sporn MB, Roberts AB, eds. Peptide growth factors and their receptors I. Vol. 95 of Handbook of experimental pharmacology. Berlin, Germany: Springer-Verlag, 1989:419-72.
21. Weiner FR, Giambrone MA, Czaja MJ, et al. Ito-cell gene expression and collagen regulation. Hepatology 1990; 11:111-7.
22. Maher JJ, McGuire RF. Extracellular matrix gene expression increases preferentially in rat lipocytes and sinusoidal endothelial cells during hepatic fibrosis in vivo. J Clin Invest 1990; 86:1641-8.
23. Matsuoka M, Tsukamoto H. Stimulation of hepatic lipocyte collagen production by Kupffer cell-derived transforming growth factor β: implication for a pathogenetic role in alcoholic liver fibrogenesis. Hepatology 1990; 11:599-605.
24. Nakatsukasa H, Nagy P, Everts RP, Hsia C-C, Marsden E, Thorgeirsson SS. Cellular distribution of transforming growth factor-β1 and procollagen types I, III and IV transcripts in carbon tetrachloride-induced rat liver fibrosis. J Clin Invest 1990; 85:1833-43.
25. Brouwer A, Wisse E, Knook DL. Sinusoidal endothelial cells and perisinusoidal fat-storing cells. In: Arias IM, Jakoby WB, Popper H, Schachter D, Shafritz DA, eds. The liver: biology and pathobiology. 2nd ed. New York: Raven Press, 1988:665-82.
26. Geerts A, Schellinck P, Wisse E. Sinusoidal liver cells and cirrhosis. In: Tygstrup N, Orlandi F, eds. Cirrhosis of the liver: methods and fields of research. Amsterdam: Elsevier, 1987:83-90.
27. Milani S, Herbst H, Schuppan D, Surrenti C, Riecken EO, Stein H. Cellular localization of Type I, III and IV procollagen gene transcripts in normal and fibrotic human liver. Am J Pathol 1990; 137:59-70.
28. Rickles R, Marashi F, Sierra F, et al. Analysis of histone gene expression during the cell cycle in HeLa cells using cloned human histone genes. Proc Natl Acad Sci U S A 1982; 79:749-53.
29. Gewirtz AM, Anfossi G, Venturelli D, Valpreda S, Sims R, Calabretta B. G_1/S transition in normal human T-lymphocytes requires the nuclear protein encoded by *c-myb*. Science 1989; 245:180-3.
30. Seshadri T, Campisi J. Repression of *c-fos* transcription and an altered genetic program in senescent human fibroblasts. Science 1990; 247:205-9.
31. Mead JE, Fausto N. Transforming growth factor α may be a physiological regulator of liver regeneration by means of an autocrine mechanism. Proc Natl Acad Sci U S A 1989; 86:1558-62.

32. Camps J, Castilla A, Civeira MP, Serrano M, Prieto J. Randomized trial of lymphoblastoid alfa interferon in chronic non-A, non-B hepatitis: effects on inflammation and fibrogenesis. J Hepatol 1989; 9:Suppl 1:S17. abstract.
33. Chirgwin JM, Przybyla AE, MacDonald RJ, Rutter WJ. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry 1979; 18:5294-9.
34. Chu M-L, Myers JC, Bernard MP, Ding JF, Ramirez F. Cloning and characterization of five overlapping cDNAs specific for the human pro α1(I) collagen chain. Nucleic Acids Res 1982; 10:5925-34.
35. Morello D, Fitzgerald MJ, Babinet C, Fausto N. *c-myc*, *c-fos*, and *c-jun* regulation in the regenerating livers of normal and H-2K/*c-myc* transgenic mice. Mol Cell Biol 1990; 10:3185-93.
36. Tso JY, Sun X-H, Kao TH, Reece KS, Wu R. Isolation and characterization of rat and human glyceraldehyde-3-phosphate dehydrogenase DNAs: genomic complexity and molecular evolution of the gene. Nucleic Acids Res 1985; 13:2485-502.
37. Iraburu M, Civeira MP, Serrano M, Morte S, Castilla A, Prieto J. Suppressor T-cell activity in chronic hepatitis B-virus infection: relationship with the presence of HBV-DNA in serum. J Med Virol 1989; 27:39-43.
38. Prieto J, Castilla A, Subirá ML, Serrano M, Morte S, Civeira MP. Cytoskeletal organization and functional changes in monocytes from patients with chronic hepatitis B: relationship with viral replication. Hepatology 1989; 9:720-5.
39. Knodell RG, Ishak HG, Black WC, et al. Formulation and application of a numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis. Hepatology 1981; 1:431-5.
40. Zern MA, Czaja MJ, Weiner FR. The use of molecular hybridization techniques as tools to evaluate hepatic fibrogenesis. In: Rojkind M, ed. Connective tissue in health and disease. Boca Raton, Fla.: CRC Press, 1990:99-122.
41. Ruwart MJ, Wilkinson KF, Rush BD, et al. The integrated value of serum procollagen III peptide over time predicts hepatic hydroxyproline content and stainable collagen in a model of dietary cirrhosis in the rat. Hepatology 1989; 10:801-6.
42. Annoni G, Weiner FR, Colombo M, Czaja MJ, Zern MA. Albumin and collagen gene regulation in alcohol- and virus-induced human liver disease. Gastroenterology 1990; 98:197-202.
43. Bissell DM, Friedman SL, Maher JJ, Roll FJ. Connective tissue biology and hepatic fibrosis: report of a conference. Hepatology 1990; 11:488-98.
44. Tsukamoto H, Towner SJ, Ciofalo LM, French SW. Ethanol-induced liver fibrosis in rats fed high fat diet. Hepatology 1986; 6:814-22.
45. French SW, Miyamoto K, Wong K, Jui L, Briere L. Role of the Ito cell in liver parenchymal fibrosis in rats fed alcohol and a high fat-low protein diet. Am J Pathol 1988; 132:73-85.
46. Okanoue T, Burbige EJ, French SW. The role of Ito cell in perivenular and intralobular fibrosis in alcoholic hepatitis. Arch Pathol Lab Med 1983; 107:459-63.
47. Minato Y, Hasumara Y, Takeuchi J. The role of fat-storing cells in Disse space fibrogenesis in alcoholic liver disease. Hepatology 1983; 3:559-66.
48. Mak KM, Leo MA, Lieber CS. Alcoholic liver injury in baboons: transformation of lipocytes to transitional cells. Gastroenterology 1984; 87:188-200.
49. Horn T, Junge J, Christoffersen P. Early alcoholic liver injury: activation of lipocytes in acinar zone 3 and correlation to degree of collagen formation in the Disse space. J Hepatol 1986; 3:333-40.
50. Hoofnagle JH, Mullen KD, Jones DB, et al. Treatment of chronic non-A, non-B hepatitis with recombinant human alpha interferon: a preliminary report. N Engl J Med 1986; 315:1575-8.
51. Thomson BJ, Doran M, Lever AML, Webster ADB. Alpha-interferon therapy for non-A, non-B hepatitis transmitted by gammaglobulin replacement therapy. Lancet 1987; 1:539-41.
52. Davis GL, Balart LA, Schiff ER, et al. Treatment of chronic hepatitis C with recombinant interferon alfa: a multicenter randomized, controlled trial. N Engl J Med 1989; 321:1501-6.
53. Di Bisceglie AM, Martin P, Kassianides C, et al. Recombinant interferon alfa therapy for chronic hepatitis C: a randomized, double-blind, placebo-controlled trial. N Engl J Med 1989; 321:1506-10.
54. Kershenobich D, Vargas F, Garcia-Tsao G, Tamayo RP, Gent M, Rojkind M. Colchicine in the treatment of cirrhosis of the liver. N Engl J Med 1988; 318:1709-13.
55. Czaja MJ, Weiner FR, Takahashi S, et al. γ-Interferon treatment inhibits collagen deposition in murine schistosomiasis. Hepatology 1989; 10:795-800.